EPA
United States
Environmental Protection
Agency

Office of Prevention, Pesticides,
and Toxic Substances

Date: March 17, 2009

SUBJECT: BE-7 Antimicrobial® - Review Of Ecotoxicity Studies In Support Of Marine Oil Water System Treatments

DP Barcode: 361163
PC Code: 014703 (sodium hypochlorite – 12.5%)

FROM: Richard C. Petrie, Agronomist, Team 3 Leader 3/17/09
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Antimicrobial Division (7510P)

THRU : Norm Cook. Chief 3/17/09
OPP/AD/RASSB
Antimicrobial Division (7510P)

TO: **Emily Mitchell, PM 32**
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The following marine ecotoxicity studies were submitted conditionally in support of BE-7 Antimicrobial® use in marine oil water system treatments:

MRID47642401 – Marine fish acute toxicity test using *Cyprinodon variegates* (sheepshead minnow) in a 96 hour daily static renewal system with 12.5% sodium hypochlorite. This study is Acceptable (Core).

MRID47642402 – Mysid shrimp acute toxicity test using *Americamysis bahia* (mysid shrimp) in a 96 hour daily static renewal system with 12.5% sodium hypochlorite. This study is Acceptable (Core).

MRID47642403 – Eastern oyster acute toxicity shell deposition test using *Crassostrea virginica* (Eastern oyster) in a 96 hour flow through system with 12.5% sodium hypochlorite. This study is supplemental but upgradable upon resolution of deviations noted in the DER.

No changes to the **Environmental Hazards** section of label 40153-1 are needed at this time.

DER's attached (3)

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** Sodium Hypochlorite 12.5% w/v **PC Code No.:** Not Provided

2. **TEST MATERIAL:** BE-7 Antimicrobial (Liquichlor 12.5%) **Purity:** 11.43 % w/w

3. **CITATION**
Author: R. MacGregor
Title: BE-7 Antimicrobial (Sodium Hypochlorite 12.5% w/v): Marine Fish (*Cyprinodon variegatus*) Definitive 96-hour Acute Toxicity Test with Daily Dosing
Laboratory: Baroid Bioassay Laboratory
Sponsor: Halliburton Energy Services, Inc. 1015 West Bois D'Arc Avenue, Duncan, Oklahoma 73536-0105
Study Report ID: S4546
Laboratory Report ID: BL-7830
MRID No.: 47642401

3. **REVIEWED BY:**

Richard C. Petrie, Agronomist
EPA/OPPTS/OPP/AD/RASSB

Signature: [signature] **Date:** 3/17/09

4. **APPROVED BY:**

Norm Cook, Chief [signature] 3/17/09
EPA/OPPTS/OPP/AD/RASSB

Signature: **Date:**

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: Sheepshead Minnow (*Cyprinodon variegatus*)
Age of Test Organism: 3 months
Definitive Test Duration: 96 hours
Study Method: Static renewal daily
Type of Concentrations: Nominal and mean measured

7. CONCLUSIONS

Nominal doses reflect what was added each day, while Post Dose values reflect the Total Residual Chlorine (TRC) values as measured in the test chamber seawater.

Results Synopsis: 96-hr LC_{50} (Post Dose TRC): 0.38 mg/L (measured) 95% C.I.: 0.225 and 0.65 mg/L
NOAEC (Post Dose TRC): 0.225 mg/L (measured)

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable (Core)

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations are considered minor as based on EPA OPPTS Guideline 850.1075, and not expected to significantly inpact test results:

- Signs of stress or injury to the fish were not specified.
- Not specified if the holding water was the same as the test dilution water.
- Not specified whether there was disease treatment prior to testing.
- Not specified whether light intensities were similar within 48 hours of testing.
- Intensity of the light and transition period of the day to night were not specified.
- Not specified if one replicate temperature was recorded hourly.
- Covering of test equipment not specified.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ Preferred freshwater species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*) ▪ Preferred saltwater species: Atlantic silverside (*Menidia menidia*) or Sheepshead minnow (*Cyprinodon variegatus*)	▪ Sheepshead Minnow (*Cyprinodon variegatus*)
Weight ▪ Juvenile fish < 3.0 g	▪ 0.092g
Length ▪ Longest not > 2x shortest	▪ 1.5 (1.3 – 1.7) cm
Supplier	▪ Baroid Bioassay Laboratory
All fish from same source and population?	▪ Yes – Galveston Bay, TX
Fish used in previous tests?	▪ No
If wild fish used, quarantined 7 days before acclimation?	▪ Hatchery reared, not applicable
Signs of stress or injury?	▪ Not Specified

B. Acclimation

Guideline Criteria	Reported Information
Acclimation Period ▪ Minimum 12 days (14 days recommended) ▪ Minimum 7 days in test dilution water	▪ Larvae hatched and held for at least 3 months under laboratory conditions prior to testing. ▪ Acclimation period was 7 days prior to test date.
Holding Water ▪ Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period)	▪ Synthetic seawater ▪ Not specified whether this was the same as test dilution water.
Disease Treatment ▪ No treatments within 48 hrs of test initiation or during test	▪ Not specified

Guideline Criteria	Reported Information
Feeding ▪ No feeding within 48 hrs of test initiation. Feed daily prior to this period.	▪ Fish were not fed within 48 hours of test initiation. ▪ Prior to testing, fish fed flake food and Artemia at least twice daily.
Pretest Mortality ▪ < 5% during acclimation; reject entire batch if > 10%.	▪ Cumulative mortality was 1% during acclimation.
Water Temperature ▪ Temperature changes should not exceed 3°C per day ▪ Hold fish minimum 7 days at test temperature prior to testing	▪ Acclimation period temperatures held at 22±1.0°C.
Background ▪ During final 48 hrs, colors and light intensities similar to testing area	▪ Tests conducted under controlled illumination cycle of 16 hours light and 8 hours dark. ▪ Not specified whether light intensities were similar within 48 hours of testing.

C. Test System

Guideline Criteria	Reported Information
Dilution Water ▪ Reconstituted water or water from natural source preferred. If dechlorinated tap water, daily chlorine analysis performed. ▪ Chemical analysis performed and maximum concentrations not exceeded (see guideline)	▪ Synthetic seawater was used and daily chlorine analysis performed. ▪ Water quality measurements were conducted twice per week.
Solutions ▪ Distilled water used to make stock solutions of test substances. If stock volume > 10% of test solution volume, dilution water used.	▪ Rangefinder: Working standard prepared with 1.0 mL of test material mixed with 125 mL deionized water was combined in an appropriate volume with 1000 mL of dilution seawater. ▪ Definitive test: The dosing solution (0.5 mL of test chemical and 624.5 mL deionized water) was added in appropriate volumes 4000 mL of dilution seawater.

Guideline Criteria	Reported Information
Water Temperature ▪ 10 or 12 ± 2°C for cold water species (see guideline) ▪ 22 or 23 ± 2°C for warm water species (see guideline) ▪ Vary no more than 1°C in any 24-hr period ▪ Record in all replicates at beginning of test and every 24 hrs; record hourly in one replicate.	▪ Temperatures held at 22±1.0°C. ▪ Temperature did not vary more than 1°C in any 24-hr period. ▪ Temperature recorded at the beginning and of test, and in all replicates at least once daily. ▪ Not specified if one replicate was recorded hourly.
pH ▪ > 6.0 and < 8.0 for freshwater testing ▪ > 7.5 and < 8.5 for marine testing ▪ Measured in each replicate at beginning of test and every 24 hrs	▪ Between 7.5-8.0 ▪ pH measured initially and every 24 hours.
Dissolved Oxygen ▪ Static: > 60% saturation at all times ▪ Flow-through: > 75% saturation at all times ▪ Measured in each replicate at beginning of test and every 24 hrs	▪ > 71% dissolved oxygen (5.2-7.7 mg/L) ▪ Dissolved oxygen measured initially and every 24 hours.
Total Hardness ▪ 40 to 180 mg/L as $CaCO_3$ (freshwater species) ▪ Measured at beginning of each test	▪ Estuarine species used.
Salinity ▪ 20 ± 5ppt (estuarine species) ▪ Measured at beginning of each test and, for flow-through tests, on day 4, and if extended days 7 and 14	▪ Salinity ranged between 20.1 and 20.6 ppt. ▪ Salinity was measured initially and every 24 hours. ▪
Test Aquaria/Equipment ▪ Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®) ▪ Test chambers loosely covered	▪ 1 gallon-capacity, wide-mouth glass jars used. ▪ Covering not specified.
Aeration ▪ Static systems only if < 60% saturation; if aeration used test concentrations measured. ▪ No aeration in flow-through tests	▪ During acclimation system supplied with gentle aeration. ▪ Aeration during testing not specified.
Type of Dilution System ▪ Must provide reproducible supply of toxicant	▪ Reproducible supply of toxicant provided. ▪ 0.1 mg NaOCL / ml dosing solution

Guideline Criteria	Reported Information
Flow Rate ▪ Consistent flow rate of 6-10 vol/24 hours ▪ Measured at beginning and end of each test ▪ No more than a factor of 10 variation between replicates	▪ Static delivery system used.
Biomass Loading Rate ▪ Static/Static-renewal: ≤ 0.8 g FWF/L ▪ Flow-through: ≤ 0.5 g FWF/L	▪ Maximum loading density was 0.8g fish/liter.
Photoperiod ▪ Range from 12D/12N to 16D/8N, with 15 min transition period ▪ Intensity 30 to 100 lm at water surface	▪ Photoperiod was 16 hours light and 8 hours dark. ▪ Intensity and transition period not specified.
Solvents ▪ Not to exceed 0.5 ml/L for static or static-renewal tests or 0.1 ml/L for flow-through tests ▪ Preferred solvents dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol	▪ No solvents used.

D. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ If LC_{50} > 100 mg/L with 30 fish, then no definitive test required	▪ Pulse-static conditions over 96-hours with five animals exposed per concentration (one replicate per concentration). Five concentrations and one control conducted. ▪ LC_{50} (nominal): 1.12 mg/L

Guideline Criteria	Reported Information
Test Concentrations ▪ Minimum of control and 5 concentrations in geometric series ▪ Concentrations 50 to 120% greater than next lowest concentration ▪ No more than 25% variation between test concentrations within same treatment ▪ Concentrations selected to produce NOEC and, preferably, at least 2 partial mortalities (> and < 50%) after 96 hrs ▪ Measured concentrations required if test chemical unstable or flow-through system, and must remain at least 80% of nominal concentrations	▪ 5 concentrations and a control were used at 0.0, 0.05, 0.10, 0.25, 0.5, and 1.0 mg/L (nominal). ▪ Used to produce LC_{50} and NOEC. ▪ Did not achieve 2 partial mortalities after 96 hours. ▪ Test material yielded significantly lower measured values as TRC when added to synthetic seawater as compared to when added to DI water; therefore, TRC values were measured in the test chamber seawater. ▪ Daily dosing was necessary because the measurable TRC levels fell rapidly by 75-90% within 24 hours; therefore, the organisms received a daily pulse of sodium hypochlorite, measured as TRC.
Concentration Analysis ▪ Performed at test initiation and every 48 hrs ▪ Static: each replicate, minimally at test initiation (before organisms added), at 48 hrs and at end of test ▪ Static-renewal: each replicate, at test initiation and end, and just before and after each renewal ▪ Flow-through: each replicate at 0, 48, and 96 hrs, and every 96 hrs thereafter	▪ Measured at initiation and every 24 hours (before and after each renewal).
Controls ▪ Consist of same dilution water, conditions, procedures and test population ▪ Negative and/or solvent ▪ Maximum allowable mortality 10% (or 1 mortality if 7 to 10 fish used) for 96 hr period; 10% additional past 96 hrs.	▪ Same dilution water, conditions and procedures as treatment groups. ▪ 0% mortality
Replicates ▪ Two per test concentration ▪ Equal volume test solution and number test fish	▪ Two replicates per test concentration at 10 fish each and 2 liters of test material for all.

Guideline Criteria	Reported Information
Test Organisms ▪ Minimum 7/replicate (10 preferred) ▪ Equal number per test chamber ▪ Not fed during treatment period ▪ Randomly or impartially assigned to test vessels within 30 min of addition of test substance ▪ Biological observations made at 6 hrs and every 24 hours	▪ Observations made every 24 hours. ▪ Specimens were not fed during treatment period. ▪ 20 fish per vessel. ▪ Equal number in test chamber except for final retest (only 10 organisms instead of 20). ▪ Randomly assigned to for testing but not specified whether it was within 30 min of addition of test substance.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	▪ Yes, pg 3-4
Name of test facilities, test dates and personnel reported?	▪ Yes, pg 1 and pg 7
Identification of test substance (including physicochemical characteristics) and purity provided?	▪ Yes, pg 20 Appendix D
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	▪ Yes, pg 16 Appendix B
LC_{50} concentration-response curves, LC_{50} values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOAEL also reported?	▪ No concentration response curve reported ▪ LC_{50} values, 95% C.I. presented for 48 and 96-hr as well as NOAEC reported, pg 11. ▪ LC_{50} values for 24 and 72-hr not reported but would be the same as the 48 and 96-hr values.
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	▪ Yes, pg 12
Any protocol deviations which may have influenced final results of test reported?	▪ Deviations reported, pg 13 but none that would influence final results of test
Raw data included?	▪ Yes, pg 43-50 Appendix F
Signs of abnormal behavior by test fish (if any) described?	▪ None

Guideline Criteria	Reported Information
Statistical methods reported?	▪ Yes, pg 15

Dose Response:

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Number of Fish at Test Initiation	Number of Dead Fish			
			24 hour	48 hour	72 hour	96 hour
Control	Control	20	0	0	0	0
0.05	0.050	20	0	0	0	0
0.1	0.066	20	0	0	0	0
0.25	0.138	20	0	0	0	0
0.5	0.225	20	0	0	0	0
1.0*	0.65	20	20	20	20	20
1.0**	0.70	10	10	10	10	10

*All fish dead in 4 hours.
**Reset test. All fish dead in 5 hours.

Statistical Results: The mean measured concentrations tested and the corresponding mortality data derived from the definitive toxicity test were used to estimate the 48- and 96-hour median lethal concentrations (LC_{50}) and 95% confidence intervals. The number of dead organisms was expressed as a proportion of the total number exposed. Where sufficient response was observed, the LC_{50} values and NOEC values were calculated from the proportional response data using the Trimmed Spearman Karber method.

Results Synopsis:

Duration	LC_{50} (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	NA	NA	NA
48-hr	0.38	0.225	0.65
72-hr	NA	NA	NA
96-hr	0.38	0.225	0.65

NA = Not available

NOAEC through 96 hours = 0.225

Other Effects Observed: A reset of the high concentration was conducted because total chlorine reading had not been made at the observed time of 100% mortality. In the first and second high concentration replicates all test organisms were observed dead at 4 and 5 hours respectively.

13. VERIFICATION OF STATISTICAL RESULTS

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             EXPOSED        DEAD           DEAD          PROB.(PERCENT)
 .65           20            20             100           9.536742E-05
 .225          20            0              0             9.536742E-05
 .138          20            0              0             9.536742E-05
 .066          20            0              0             9.536742E-05
 .05           20            0              0             9.536742E-05

THE BINOMIAL TEST SHOWS THAT .225 AND .65 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.


AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .3824265


WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

************************************************************************
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
? _
```

Statistical Method: Binomial Test

Results Verification Synopsis: 96-hr LC_{50} (analytically measured): 0.3824265 95% C.I: 0.225 and 0.65 mg/L
NOAEC: Not Available

14. REVIEWER'S COMMENTS: The study seemed adequate for an acute toxicity study, but did have some minor guideline deviations. Some guideline deviations considered minor include discussion of the pre-test acclimation period, whether the holding water was the same as the test water, whether the lighting was similar to testing conditions, and whether or not the test organisms underwent disease treatment prior to testing. These were not considered to influence the results of the study.

DATA EVALUATION RECORD
MYSID ACUTE TOXICITY TEST
GUIDELINE OPPTS 850.1035

1. **CHEMICAL:** Sodium Hypochlorite 12.5% w/v **PC Code No.:** Not Provided

2. **TEST MATERIAL:** BE-7 Antimicrobial (Liquichlor 12.5%) **Purity:** 12.5% w/v

3. **CITATION**

Author: R. MacGregor
Title: Mysid Shrimp (*Americamysis bahia*) Definitive 96-hour Acute Toxicity Test With Daily Dosing.
Study Completion Date: March 25, 2008
Laboratory: Halliburton Energy Services
Baroid Bioassay Laboratory
3000 No. Sam Houston Parkway East
Houston, TX 77032
Sponsor: Halliburton Energy Services
1015 W. Bois D'Arc Ave.
Duncan, OK 73536-0105
Study Report No.: S4547
Laboratory Report ID: BL 7831
MRID No.: 47642402

4. **REVIEWED BY:**

Richard C. Petrie, Agronomist
EPA/OPPTS/OPP/AD/RASSB

Signature: Richard C. Petrie **Date:** 3/17/09

5. **APPROVED BY:**

Norm Cook, Chief
EPA/OPPTS/OPP/AD/RASSB

Signature: Norm Cook **Date:** 3/17/09

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Americamysis bahia*
Age of Test Organism: Juvenile (≤ 24-hr old)
Definitive Test Duration: 96 hours
Study Method: Static renewal daily
Type of Concentrations: Nominal and Measured Post Dose Total Residual Chlorine (TRC)

7. CONCLUSIONS

Results Synopsis: Mean Measured Post Dose TRC

96-hr LC_{50}: 0.16 mg/L 95% C.I.: 0.14-0.17 mg/L

NOAEC: 0.14 mg/L

8. ADEQUACY OF THE STUDY

A. Classification: ACCEPTABLE (Core)

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1035:

- The report states that there was no pre-test acclimation period (Appendix H, Page 4), however, it is assumed that acclimation occurred based on the statement "the shrimp were fed twice daily during acclimation".
- Report did not explicitly mention if glass test chamber was covered.
- There was no mention of a 15 to 30 minute transition period between the 14 hours of light and the 10 hours of dark photoperiod.
- Study report did not explain how artificial seawater was prepared.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ Mysids (*Mysidopsis bahia*; now *Americamysis bahia)*	• *Americamysis bahia*
▪ Life Stage/Size Should be either juvenile (<24 hours old) or young adults (5 to 6 days old) ▪ Should be of normal size and appearance for their age.	• Rangefinder test period: o Group 1: ≤ 24-hr old o Group 2: 6 days • Definitive Test Period: ≤ 24-hr old • No information on size or appearance provided.

Acquisition ▪ Mysids should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. ▪ Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. ▪ Taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.	▪ The Baroid Bioassay Laboratory has maintained the culture stock of *Americamysis bahia* for 5 years. Adults from other laboratories are introduced occasionally to increase genetic variation.
Acclimation ▪ Within a 24–h period, changes in water temperature should not exceed 1°C, while salinity changes should not exceed 5 percent. ▪ During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.	▪ Post-larvae are collected dialy and held under laboratory conditions prior to testing. ▪ Mysids were fed twice daily during acclimation. ▪ Daily batches of mysids are held under semi-static conditions in 10-L capacity polyethene taks, containing synthetic seawater and supplied with gentle aeration and recirculating water treatment.

B. Test System

Guideline Criteria	Reported Information
Test Chamber ▪ Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results. ▪ Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions. ▪ For flow through tests, retention chambers can be constructed with netting material of appropriate mesh size	▪ Testing was conducted in wide mouth glass jars with a 1-L capacity. ▪ Study report did not indicate whether test chambers were covered.
Test Substance Delivery System (flow-through tests) ▪ Proportional diluters, metering pumps, or other suitable systems should be used. ▪ System should be calibrated prior to start of test. ▪ General operation should be checked 2x daily. ▪ 24-hr flow through a test chamber should be equal to 5x the volume of the test chamber. ▪ Flow rates among chambers or across time should not vary more than 10%.	▪ Experimentation was conducted under static non-renewable conditions; however, 24-hr pulse additions of test material due to rapid degradation was needed. ▪ The initial preparations were mixed in situ, before introduction of test organisms to ensure adequate dissolution.

Temperature ▪ The test temperature should be 25°C. ▪ Deviations from the test temperature should be not greater than ±2°C. ▪ Measured in each chamber at beginning and end of test	▪ Temperatures ranged between 23.9 and 24.3°C and was measured in one replicate at each concentration every 24 hours.
pH ▪ Measured in each chamber at beginning and end of test	▪ pH ranged from 7.8-8.1 and was measured in one replicate at each concentration before and after the renewal of media.
Salinity ▪ Salinity of 20 ± 3 ppt. ▪ Measured in each chamber at beginning and end of test	▪ Salinity was measured in one replicate at each concentration before and after the renewal of media. ▪ Salinity, during testing conditions, ranged between 20.2 and 20.8 ppt.
Dissolved Oxygen ▪ Dissolved oxygen concentration between 60 and 105 % saturation. ▪ Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment. ▪ Measured in each chamber at beginning and end of test	▪ Dissolved oxygen was measured in one replicate at each concentration before and after the renewal of media. ▪ Dissolved oxygen, during testing conditions, ranged from 6.1 to 7.7 mg/L, which was greater than the required minimum 4.4 mg/L saturation.
Photoperiod ▪ Photoperiod of 14 hours light and 10 hours dark, with a 15 to 30 min transition period.	▪ Tests were conducted under controlled photoperiod of 14 hours light and 10 hours dark. ▪ No mention of a transition period in the study report..
Feeding ▪ Mysids should be fed daily during testing. ▪ A recommended food is live Artemia spp. (48–h–old nauplii).	▪ Mysids were fed a satiating amount of <24-hr old *Artemia* once daily.

Dilution Water ▪ Natural seawater or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating, and testing periods without showing signs of stress. ▪ Mysids should be cultured and tested in dilution water from the same origin. ▪ Natural seawater should be filtered through a filter with a pore size of <20 Φm prior to use in a test. ▪ Artificial seawater can be prepared by adding commercially available formulations or specific amounts of reagent-grade chemicals to deionized water (conductivity should be <1 Φohm/cm at 12°C). ▪ If artificial seawater prepared from ground or surface water source, conductivity and total organic carbon should be measured.	▪ Synthetic seawater was used during experimentation. ▪ The study did not detail how the synthetic seawater was prepared. ▪ Not reported whether mysids were cultured and tested in dilution water from the same origin.
Test Substance ▪ Concentration of test substance should be measured: --at beginning and end of test for static tests --at beginning and end of test and in at least one appropriate chamber whenever a malfunction is detected for flow-through tests ▪ Among replicate test chambers, measured concentrations should not vary by more than 20%.	▪ TRC was measured daily, in each replicate, before and after daily dosing with test material. TRC was measured again when 100% mortality was observed within the first 24 hours of exposure.
Carriers ▪ Use of carriers should be avoided, if possible, as they may serve as a carbon source for bacteria. ▪ If solvents, solubilizing agents, or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. Preferred carriers are dimethyl formamide, triethylene glycol, acetone, or ethanol. ▪ Concentration of carriers should not exceed 0.1 mL/L	• No carriers were used in this testing scenario.

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ Range finding test should be conducted to determine (1) which life stage (juvenile or young adult) is to be utilized in the definite test; and (2) the test solution concentrations for the definite test. ▪ The mysids should be exposed to a series of widely spaced concentrations of test substance (e.g. 1, 10, 100 mg/L, etc.), usually under static conditions. ▪ A minimum of 10 mysids for each age class (juvenile or young adult) should be exposed to each concentration of test substance for up to 96 hours. The age class which is most sensitive to the test substance in the range-finding test should be utilized in the definitive test.	▪ The range finding test was conducted from January 29-Febrary 2, 2008. ▪ Range finding tests were conducted under static conditions over 96-hours at concentrations of 0, 0.2, 0.6, 1.0, or 2.0 mg/L. Ten organisms were exposed per concentration level with one or more replicates. ▪ Two range finding tests were conducted, one with ≤ 24-hr old mysids and the other with 6-day old mysids.
Doses ▪ At least 5 test concentrations should be used. ▪ Dilution factor between concentration should be chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L).	▪ Definitive tests were conducted under static conditions over 96-hours with thirty mysids exposed in 2 replicates per concentration (15 mysids per replicate, 30 mysids total per test concentration). ▪ The organisms were exposed to nominal concentrations of 0, 0.1, 0.2, 0.4, 0.6, or 1.0 mg/L. ▪ In addition to the test material concentration, daily dosing was necessary because the measurable TRC levels fell rapidly by 75-100% after 24-hrs. Daily pulses of sodium hypochlorite dosing solution were added at 0.5, 1.0, 2.0, 3.0, and 5.0 ml into 0.500 liters of test solution. ▪ The average measured post dose TRC concentrations levels were 0.01, 0.06, 0.14, 0.18, and 0.45 mg/L due to degradation of the test material.
Controls Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.	▪ Controls of the same dilution water, conditions, and procedures were used in the range finding and definitive tests.
Replicates Per Dose Two or more replicates.	▪ 2 replicates per concentration in the definitive study.

Number and Placement of Organisms: ▪ A minimum of 20 mysids per concentration. ▪ Impartially distributed among test chambers. ▪ Test chambers within the testing area should be positioned in a random manner. ▪ An equal number of mysids should be introduced into the test and control chambers. ▪ Loading of mysids in static tests should not exceed 30 mysids per liter ▪ Loading for flow-through tests will vary depending on flow rate	▪ 15 mysids were exposed per concentration per replicate in the definitive study, 30 mysids total per test concentration. ▪ Mysids were randomly selected for testing from the appropriate age batch. ▪ The maximum loading density for testing was about 20 mysids/0.5 liter, this exceeds EPA's recommended loading density.
Duration of Test ▪ 96 hours	▪ 96-hours, with mortality monitored and recorded every 24-hour.
Endpoints ▪ mortality	▪ mortality

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	▪ Included
The nature of the test, laboratory, name of the investigator, test substance, and dates of test reported?	▪ Included
Control ▪ Mortality should not exceed 10% at end of test	▪ Mortality does not exceed 10% at end of test
Detailed description of the test substances (e.g. the source, lot number, composition, physical and chemical properties, shelf life and storage conditions, and any carrier or additives used)?	▪ Included
Detailed information about the shrimp (e.g. common and scientific names, source of supply, age, history, weight, acclimation procedure, and feeding history)?	▪ Partially included. No information on weight was mentioned. There was no pre-test acclimation period.
A description of the experimental design including the number of test solution concentrations, number of replicates, and number of shrimp per replicate?	▪ Included
The source of the dilution water, its chemical characteristics (e.g. salinity), and a description of any pretreatment?	▪ Not included.
A description of the test chambers, the depth and volume of solution in the chamber, the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and flow rate expressed as volume additions per 24 h?	▪ Included
The concentration of the test substance in each test chamber before the start of the test and at the end?	▪ Included

Number of dead shrimp and measurements of water temperature, salinity, and dissolved oxygen concentration in each test chamber recorded at the protocol-designated times?	▪ Included
Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks?	▪ Included
Recorded data for the holding and acclimation period (temperature, salinity, etc.)?	▪ Mysids were fed twice daily during acclimation.
Concentration-response curves should be fitted to mortality data collected at 24, 48, 72, and 96 h. A statistical test of goodness-of-fit performed?	▪ Included
For each set of mortality data, the 48– and 96–h LC50 and 95 percent confidence limits calculated on the basis of the average measured concentration of the test substance. When data permits, LC50 values with 95 percent confidence limits should be computed for 24– and 72–h observations. The NOAEC and slope of the dose-response curves calculated?	▪ Included
The methods used in calculating the concentration-response curves and the LC50 values should be fully described?	▪ Methods used in calculating the concentration-response curves and LC50 values were not described in study report.

Dose Response

Nominal Concentration (mg ai/L)	Mean Measured Post Dose TRC	Mortality after 96 hours		% Mortality (96 hour)
		Replicate A	Replicate B	
1.0	0.45	15	15	100
0.6	0.18	15	12	90
0.4	0.14	1	0	3
0.2	0.06	0	1	3
0.1	0.01	0	0	0
Control	Control	0	0	0

Statistical Results

Statistical Method: The nominal and mean measured concentrations tested and the corresponding mortality data were used to estimate LC_{50} and 95% confidence intervals (CIs). Study authors used the Trimmed Spearman-Karber Method to calculate the LC_{50}, 95% C.I., and the NOAEC (Appendix E, page 29) for each set of concentrations

Results Synopsis:

For Nominal Concentrations			
Duration	**LC_{50} (mg a.i./L)**	**95% Lower CI**	**95% Upper CI**
24-hr [a]	> 1.0	--	--
48-hr	0.77	0.74	0.81
72-hr	0.59	0.53	0.66
96-hr	0.49	0.46	0.53

NOEC through 96 hours = 0.40 mg/L

[a] The 24-hr survival was >86% in all concentrations. Therefore, no LC_{50} was calculated. Values in chart are what LC_{50} values would be.

For Mean Measured Post Dose TRC			
Duration	**LC_{50} (mg a.i./L)**	**95% Lower CI**	**95% Upper CI**
24-hr[a]	> 0.50	--	--
48-hr	0.28	0.26	0.31
72-hr	0.19	0.16	0.22
96-hr	0.16	0.14	0.17

NOEC through 96 hours = 0.14 mg/L

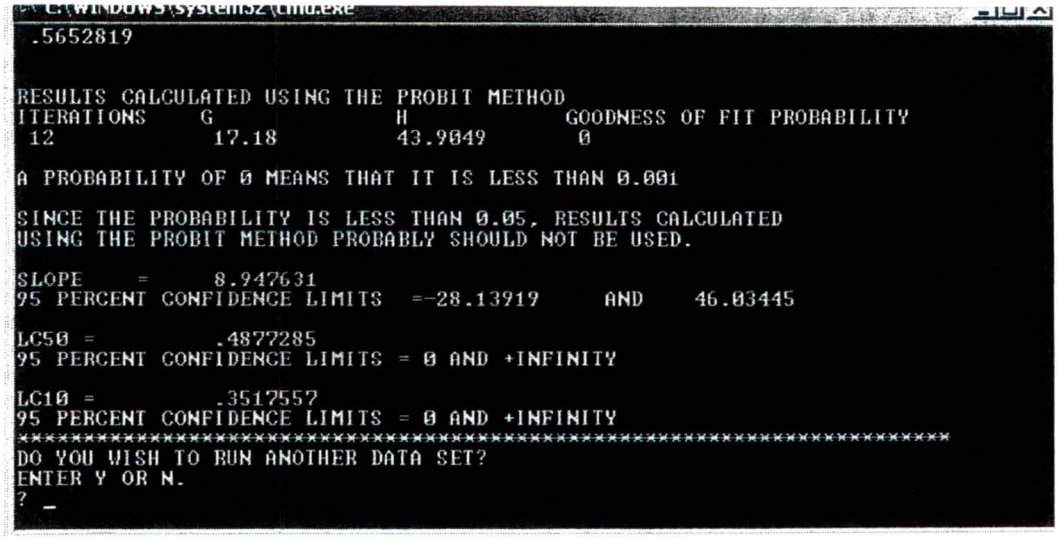
[a] The 24-hr survival was >86% in all concentrations. Therefore, no LC_{50} was calculated. Values in chart are what LC_{50} values would be.

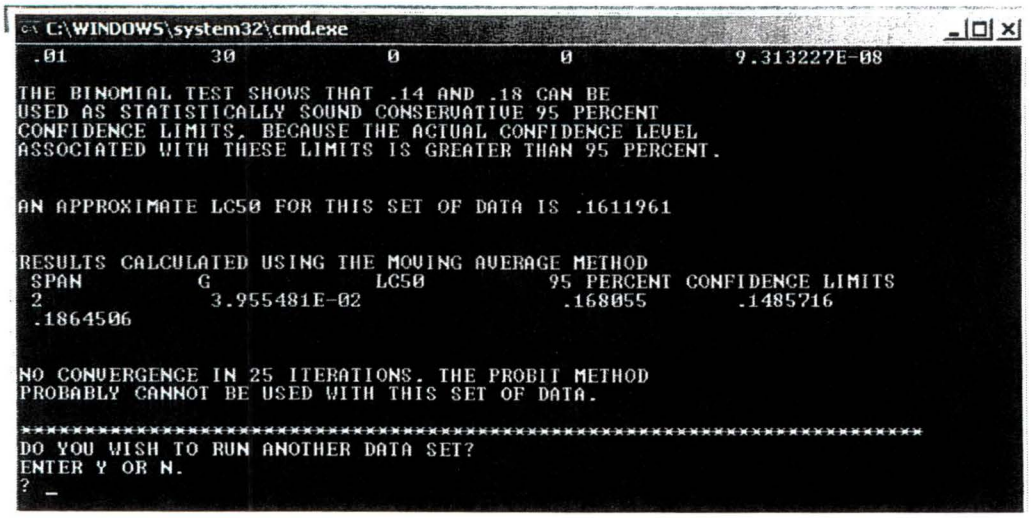
13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

Results Verification Synopsis: Nominal Values
96-hr LC_{50}: 0.49 mg/L 95% C.I.: Not Available
NOAEC: Not Available

Post Dose TRC
96-hr LC_{50}: 0.16 mg/L 95% C.I.: 0.14-0.18 mg/L
NOAEC: Not Available





14. **REVIEWER'S COMMENTS:** In general, this study meets the EPA guidelines for testing acute toxicity in mysid shrimp. There were some minor guideline deviations that are not believed to have significantly impacted test results.

DATA EVALUATION RECORD
OYSTER ACUTE TOXICITY TEST (SHELL DEPOSITION)
OPPTS GUIDELINE 850.1025

1. **CHEMICAL:** Sodium Hypochlorite 12.5% w/v **PC Code No.:** Not Provided

2. **TEST MATERIAL:** BE-7 Antimicrobial (Liquichlor 12.5%) **Purity:** 12.96%

3. **CITATION**

Author:	S. P. Gallagher, T. Z. Kendall, H. O. Krueger
Title:	BE-7 Antimicrobial (Sodium Hypochlorite 12.5% w/v): A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
Study Completion Date:	January 6, 2009
Laboratory:	Wildlife International, LTD.
Sponsor:	Haliburton Energy Services, Inc.
Laboratory Report ID:	666A-101
MRID No.:	47642403

4. **REVIEWED BY:**

Richard C. Petrie, Agronomist
EPA/OPPTS/OPP/AD/RASSB

Signature: [signature] **Date:** 3/17/09

5. **APPROVED BY:**

Norm Cook, Chief
EPA/OPPTS/OPP/AD/RASSB

Signature: [signature] **Date:** 3/17/09

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Crassostrea virginica*
Valve Height:	Mean: 41.6 ± 3.74 mm Range: 35.1 – 48.6 mm
Definitive Test Duration:	96-hr
Study Method:	Flow-through
Type of Concentrations:	Nominal and mean measured

7. CONCLUSIONS

Results Synopsis:

96-hour EC_{50} (moving average) = 0.20 mg a.i./L (nominal)
95% C.I. = 0.14 - 0.27 mg a.i./L

NOAEC = 0.13 mg a.i./L (nominal)

Results are based on Nominal concentrations because the mean measured concentrations were below the limit of quantification for 4 of 5 test concentrations.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: Mean measured concentrations were too low for a flow through study.

C. Repairability: Upgradable depending on resolution of deviations below.

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1025:

- No information provided on spawning, absence of gametes in gonadal tissue, or prespawn condition.
- Nominal test concentrations were used to plot concentration-response curve and to calculate EC50 and 95 percent confidence limits. Mean measured concentrations should have been maintained throughout the test in a flow through system.

Nominal mg ai/L	Mean measured mg ai/L
0.063	< LOQ*
0.130	< LOQ
0.250	< LOQ
0.500	< LOQ
1.000	0.230
	* LOQ = 0.103 mg ai/L

- Eleven-day holding period preceding test less than guideline requirement.
- No information provided for mean, standard deviation, and range of salinity, pH, temperature, and dissolved oxygen during the test period.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Species ▪ Eastern oyster - *Crassostrea virginica*	▪ Eastern oyster - *Crassostrea virginica*
Life Stage/Size ▪ 30-50 mm in valve height ▪ As similar in age and/or size as possible to reduce variability ▪ Standard deviation of valve height < 20% of the mean ▪ Should be in pre-spawn condition of gonadal development prior to and during test as determined by direct or histological observation of the gonadal tissue for presence of gametes	▪ Mean valve height of 41.6 ± 3.74 mm (pp. 8 and 11) ▪ Range: 35.4 – 48.6 mm in valve height (pp. 8 and 11) ▪ No information provided for evidence of pre-spawn condition
Acquisition ▪ Oysters may be cultured in laboratory, purchased from culture facilities or commercial harvesters, or collected from a natural population in an unpolluted area free from epizootic disease	▪ Received from Circle C Oyster Ranch, Ridge, MD (p. 10)
Acclimation ▪ Upon receipt, oysters brushed clean of fouling organisms and gradual transfer of oysters to holding water ▪ Oysters held for at least 12-15 days before testing ▪ Oyster held in dilution water at test temperature for at least 2 days before used ▪ During holding, dissolved oxygen > 60% saturation and temperature of holding water should be same as testing water ▪ Batch of oysters acceptable for testing if mortality over 7-day period prior to testing is <5%; if mortality between 5 and 10%, acclimation should continue for 7 additional days; if mortality >10%, entire batch should be rejected ▪ Oysters which appear diseased, have cracked, chipped, bored or gaping shells, or are infested with mudworms or boring sponges should not be used	▪ No information provided for cleaning and transfer of oysters to holding water ▪ Held for 11 days prior to testing (p. 11) ▪ Held in dilution water at temperatures ranging from 19.7 to 22.3 (p. 11) ▪ Dissolved oxygen concentration ≥ 91% saturation(p. 11) ▪ No observed signs of disease or stress during holding period (p. 11)

Guideline Criteria	Reported Information
Preparation for Definitive Test ▪ Oysters which have been acclimated and meet condition criteria should have 3-5 mm of the shell periphery, at the rounded end, ground away with a small electric disc grinder or other appropriate device to produce a smooth, rounded, blunt profile ▪ Oyster's valves should be held together tightly during grinding to avoid vibrating shell and injuring abductor muscle ▪ If an opening into the shell cavity is visible, oyster should not be used	▪ Removed recently deposited shell with an electric grinder (p. 11)

B. Test System

Guideline Criteria	Reported Information
Test Chamber ▪ Tanks should be made of chemically inert material	▪ 54 L glass aquaria used (p. 12)
Temperature ▪ Test temperature should be 20°C ▪ Temporary fluctuations (less than 8 hr) within ± 5°C permissible ▪ Should be recorded continuously	▪ 20 ± 2°C (p. 16) ▪ Measured at test initiation and termination in each test chamber (p. 15) ▪ Measured continuously in negative control test chamber (p. 15)
Salinity ▪ Dilution water – salinity in excess of 12 ppt ▪ Natural seawater – weekly range of <10 ppt ▪ Artificial seawater – should not vary by more than 2 ppt ▪ Measured at beginning and end of test in each chamber.	▪ Dilution water – salinity of 20ppt (p. 23) ▪ Measured at beginning and end of test in each test chamber (p. 15)
Dissolved Oxygen ▪ Dissolved oxygen concentrations should be at least 60% during and at the end of the test. ▪ Measurements should be made daily from the beginning to the end of the test in each chamber	▪ ≥ 91% (p. 23) ▪ Measured in each test chamber at initiation and then in 24-hour intervals (p. 15)

Guideline Criteria	Reported Information
pH ▪ Measured in each test chamber at the beginning and end of test. ▪ Dilution water should have a monthly range of <0.8 unit ▪ Artificial seawater pH should not vary by more than 0.5 unit ▪ Test should be carried out without adjustment of pH unless there is evidence of marked change, in which case the guidelines advise that test be repeated with pH adjustment to dilution water	▪ Measured at test initiation, midpoint and termination (p. 15) ▪ Dilution water ranged from 8.1 to 8.1 during 4-week period preceding test (p. 27) ▪ Ranged from 8.0 to 8.2 during test (p. 23)
Feeding ▪ Cultured algae may be added to dilution water sparingly as needed	▪ Suspension of marine microalgae provided at a nominal rate of 2.9 x 10^9 cells/oyster/day and during holding and at a nominal rate of 5.8 x 10^9 cells/oyster/day during test (p. 11)
Dilution Water ▪ Constant supply of good quality unfiltered seawater should be available throughout holding, acclimation, and testing periods ▪ Natural seawater recommended, but artificial seawater with food added can be used ▪ Should be delivered at a flow rate of at least 1 and preferably 5 L/hr/oyster ▪ Flowrate should be ±10% of nominal flow ▪ Dilution water is acceptable if oysters survive and grow normally for 14 days without exhibiting signs of stress	▪ Filtered and diluted natural seawater collected at Indian River Inlet, Delaware with added food (p. 11) ▪ Delivered at flow rate of at least 1 L/hr/oyster (p. 12) ▪ Oysters held in dilution water for 11 days without exhibiting signs of stress (p. 11)
Carriers ▪ Stock solutions of test substances of low aqueous solubility may be prepared by ultrasonic dispersion or by use of organic solvents, emulsifiers, or dispersants of low toxicity to oysters ▪ When used, control oysters should be exposed to same concentration of carrier as that used in highest concentration treatment ▪ Concentration of carriers should not exceed 0.1 mL/L	▪ No information provided on carriers

C. Test Design

Guideline Criteria	Reported Information

Range-Finding Test ▪ Should be conducted to establish test chemical concentrations for the definitive test ▪ Test should be conducted in same way as definitive test except a widely spaced concentration series (i.e., log-interval) is used	▪ Nominal test concentrations were based on results of exploratory range finding toxicity data and selected in consultation with Sponsor (p. 9) ▪ No information provided on conducting range-finding test
Doses ▪ At least 5 test concentrations should be used ▪ Dilution factor between concentration should not exceed 1.8 ▪ Test chemical concentrations should be documented	▪ 5 concentrations and one control (p. 9) ▪ Dilution factor between concentrations ≈1/2 ▪ Nominal [control, 0.063, 0.13, 0.25, 0.50, 1.0 mg a.i./L] (p. 9)
Number and Placement of Organisms: ▪ 20 oysters per test chamber. ▪ Impartially distributed among test chambers ▪ Spread out equidistantly from one another with left (cupped) valve down and open, unhinged ends all oriented in same direction facing the incoming flow of test solution	▪ 20 oysters per test chamber (p. 11) ▪ Indiscriminately distributed among test chambers ▪ Placed in chamber with flat valves facing up and umbos away from the flow of the water (p. 11)
Duration of Test ▪ 96 hours	▪ 96 hours (p. 17)
Endpoints ▪ Shell growth	▪ Shell growth
Shell Growth Measurements ▪ Oysters should be inspected at least after 24, 48, 72, and 96 hours ▪ Dead oysters should be removed ▪ Shell growth increments measured after 96 hours ▪ Record the length of the longest "finger" of new shell growth to the nearest 0.1 mm	▪ Inspected daily for mortality and other signs of toxicity (p. 17) ▪ Shell growth measured after 96 hours ▪ Shell growth recorded to the nearest 0.1 mm

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	▪ Quality Assurance Statement on page 4 ▪ GLP Compliance Statement on page 3
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	▪ Provided on title page and page 5
Control ▪ Mortality should not exceed 10% at end of test ▪ Minimum of 2 mm of new shell growth should be observed	▪ 0% mortality (p. 24) ▪ 3.5 ± 1.6 mm new shell growth (p. 25)
Information on test chemical (e.g., water solubility, vapor pressure, purity, stability in water and light, n-octanol/water partition coefficient, and pKa values)?	▪ No information provided on test chemical

Guideline Criteria	Reported Information
Source of dilution water, the mean, standard deviation and range of salinity, pH, temperature, and dissolved oxygen during test period?	▪ Dilution water was filtered natural seawater collected at Indian River Inlet, Delaware and diluted with well water (p. 11) ▪ No information provided on the mean, standard deviation and range of salinity, pH, temperature, and dissolved oxygen during test period ▪ Salinity and pH of dilution water for the 4 weeks prior to the test were provided on page 27.
Description of test procedures used (e.g., flow-through system, test chambers, chemical delivery system, aeration, etc.)?	▪ Provided on pages 12-13
Detailed information on oysters used, including the age and/or size, source, history, method of confirmation of pre-spawn condition, acclimation procedures, and food used?	▪ Size and Source provided on page 8 ▪ Acclimation provided on page 11 ▪ Food used provided on page 11 ▪ No information provided on confirmation of pre-spawn condition.
Number of organisms tested, loading rate, and flow rate?	▪ 120 organisms tested, 20 in each of 6 groups (p. 9) ▪ Loading rate of one to two oysters at a time (p. 11) ▪ Flow rate $\geq$ 1L/oyster/hour (p. 12)
Methods of preparation of stock and test solutions, and test chemical concentrations used?	▪ Provided on page 13
Number of dead and live test organisms, the percentage of organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period?	▪ Provided on page 17 and Table 5 (p. 24)
96 hr shell growth measurements of each oyster, the mean, standard deviation and range of measured growth at 96 hr of oysters in each concentration of test substance and control?	▪ Measurements for each oyster provided in Appendix 4 (p. 36) ▪ Range provided in Appendix 4 (p. 36) ▪ Mean and Standard deviation provided in Appendix 4 (p. 36) and in Table 6 (p. 25)
Calculated 96 hr EC50 and its 95% confidence limits and statistical methods used to calculate values?	▪ 96 hr EC50 and its 95% confidence limits provide on pages 8, 18, and Table 6 (p. 25) ▪ Statistical methods provided on pages 15-16
Graph of concentration-response curve based on 96 hr chemical concentration and shell growth measurements upon which EC50 calculated?	▪ Provided in Figure 1 (p. 26)
When observed, the 96 hr NOAEC?	▪ NOAEC provided on page 18
Raw data included?	▪ Raw data provided in Appendix 4 (p. 36)
Methods and data records reported?	▪ Reported in Appendices
Statistical methods reported?	▪ Reported on pages 15-16

Dose Response

Nominal Concentration (μg ai/L)	Mean Measured Concentration (μg ai/L)	Treatment Mean Shell Deposition (mm)	Shell Growth Inhibition (relative to negative control)
Control	Control	3.5 ± 1.6	--
63	< LOQ[a]	2.7 ± 1.3	23
130	< LOQ	3.0 ± 1.8	14
250	< LOQ	1.4 ± 1.3*	61
500	< LOQ	0.5 ± 1.0*	87
1000	230	0.0 ± 0.0*	100

* Statistically significant difference from the negative control (using Bonferroni t-test)

[a] The limit of quantification (LOQ) was 103 μg a.i./L

Statistical Results

Statistical Method:

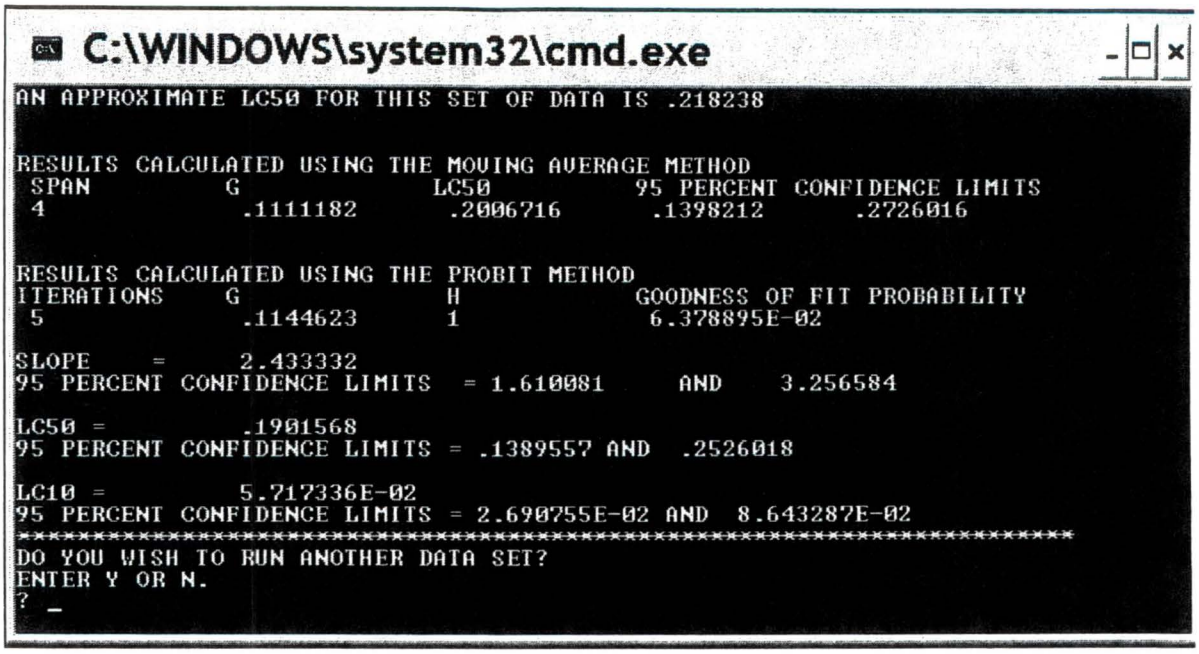
The nominal exposure concentrations and the corresponding shell deposition data derived from the definitive 96-hr test were used to statistically estimate the median effect concentration (EC50). The 96-hr EC50 value and 95% confidence interval were determined by fitting the transformed (growth data as percent reduction transformed to probit and concentrations transformed to log concentration) data using linear interpolation. The data were evaluated for normality and homogeneity of variance using Chi-Square test and Levene's test, respectively. The data were transformed with a square root transformation and then compared to the negative control data using analysis of variance (ANOVA) and the Boferroni t-test to identify significant differences. The No-Observed-Adverse-Effect-Concentration (NOAEC) was determined from statistical analysis of the data and an assessment of the concentration-response pattern.

Results Synopsis:

96-hour EC_{50} =	0.22 mg a.i./L
95% C.I. =	0.17 - 0.30 mg a.i./L
No-Mortality Concentration =	1.0 mg a.i./L
NOAEC =	0.13 mg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Moving Average Method



Results Verification Synopsis:

96-hour EC_{50} =	0.20 mg a.i./L
95% C.I. =	0.14 - 0.27 mg a.i./L
NOAEC =	Not available

14. REVIEWER'S COMMENTS:

The study seemed adequate for an acute toxicity study, but did have some limitations as described in the guideline deviations section. This study used nominal concentrations to determine EC50, 95 % confidence levels, and NOAEC because the mean-measured concentrations were below the limit of quantification in four of the five test concentrations. To verify the results using ToxAnal, Shell Growth Inhibition (percent) was multiplied by number of oysters per test group (20) and the result was rounded to the nearest whole number. (For example, 61% inhibition x 20 oysters = 12.2, rounded to 12 as input for ToxAnal). The resulting EC50 (moving average) was 0.20 mg a.i./L, which is in the range of the EC50 value given in the study. The 95% confidence interval differed from the study because rounding was required to input integer values into ToxAnal. ToxAnal calculations were 0.14 - 0.27 mg a.i./L and the study calculations were 0.17 – 0.30 mg a.i./L. This study may be upgraded pending further explanation of measured concentrations obtained.